Integrins and cancer

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The past year or two has seen great advances in the elucidation of significant roles for integrins in cancer cells. These include roles in signal transduction, gene expression, proliferation, apoptosis regulation, invasion and metastasis, and angiogenesis. In particular, integrin αvβ3 has been implicated in the neovascularization of tumors. In addition, this integrin has been shown to contribute to the survival, proliferation and metastatic phenotype of human melanoma.

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Current Opinion In Cell Biology 1996, 8:724–730

Abbreviations

bFGF	basic fibroblast growth factor
CAM	chorioallantoic membrane
FAK	focal adhesion kinase
MAPK	mitogen-activated protein kinase
RGD	Arg–Gly–Asp

Introduction

Although integrins were originally characterized as a family of cell surface receptors that are responsible for anchoring cells to the extracellular matrix, they have recently been shown to impact on such dynamic processes, in normal and tumor cells, as intracellular signaling and gene expression that leads to cell migration, proliferation, differentiation and survival. The integrin family is composed of 15 α and 8 β subunits that are contained in over twenty different αβ heterodimeric combinations on cell surfaces. Integrins bind to extracellular matrix proteins or cell surface Ig family molecules through short peptide sequences present in the ligands. Although some integrins selectively recognize a single extracellular matrix protein ligand (e.g. α5β1 integrin recognizes only fibronectin), others bind two or more ligands [1,2]. Several integrins recognize the tripeptide Arg–Gly–Asp (RGD) [1–3], whereas others recognize alternative short peptide sequences [1]. Combinations of different integrins on cell surfaces allow cells to recognize and respond to a variety of different extracellular matrix proteins.

Integrins mediate cellular adhesion to, and migration on, the extracellular matrix proteins found in intercellular spaces and basement membranes [1,2], but they also regulate cellular entry into, and withdrawal from, the cell cycle [4,5•,6]. Ligation of integrins by their extracellular matrix protein ligands induces a cascade of intracellular signals [7] that include tyrosine phosphorylation of focal adhesion kinase, increases in intracellular Ca^{2+} levels, inositol lipid synthesis, synthesis of cyclins [4], and expression of immediate early genes [5•]. In contrast, prevention of integrin–ligand interactions suppresses cellular growth or induces apoptotic cell death [5•,8–10,11•]. Thus, integrins play roles in a number of cellular processes that impact on the development of tumors, including the regulation of proliferation and apoptosis, cellular motility and invasion, cell surface localization of metalloproteinases, and angiogenesis (or the development of the vasculature that is an essential feature of solid tumor cancers). This review will focus on several of the key recent findings implicating integrin function in tumor proliferation, invasion and angiogenesis.

Integrins mediate signal transduction

Integrin ligation regulates biological events such as the survival, motility and proliferation of normal and tumor cells. Central to the many roles that integrins play in cancer are integrin-mediated signal transduction processes. Integrins transduce signals across the membrane upon ligation either by substrates such as fibronectin or by cross-linking with anti-integrin antibodies [12–14]. Among the integrin-generated signals identified to date are increases in intracellular pH [13–16], intracellular calcium [17–19], inositol lipid synthesis [20], and tyrosine phosphorylation of a tyrosine kinase associated with focal contacts, pp125 FAK (focal adhesion kinase) [21,22], in addition to activation of p34/cdc2 [23] and cyclin A [4]. Recently, the integrin-mediated activation of protein kinase C [24], mitogen-activated protein kinase (MAPK) [25•,26,27], phosphatidylinositol 3-kinase [28,29], p21Ras [30], and NF-κB [31] has also been demonstrated. Many of these signaling events can be induced directly by cross-linking of integrins on cell surfaces using specific monoclonal antibodies, suggesting that integrins alone, without accessory molecules, are responsible for these events.

The role of integrins in tumor cell proliferation

Abnormal cellular growth is one of the hallmarks of all tumors. It is now known that defects in some of the molecules that regulate the cellular proliferation machinery are common in tumor cells. Although the regulation of cellular proliferation is a complex process which requires the activities of growth factor receptors, kinases, cyclins, transcription factors and other molecules, normal cells can be induced to withdraw from the cell cycle simply by placing them in suspension [6]. Integrins on tumor cells are now thought to play intricate roles in the progression of solid tumors. Normal diploid cells can be induced to withdraw from the cell cycle and to become

quiescent by maintenance in anchorage-independent conditions [6]. They are dependent on anchorage not only for growth [6], but also for survival [8,10,11•]. In contrast to normal cells, transformed cells are characterized by their anchorage-independent growth.

The anchorage-independent growth of tumor cells may result from a transformation-associated uncoupling of cell cycle dependence on signals that are transduced by integrin-mediated attachment to the substratum [4]. Adhesion proteins have been associated with the regulation of growth since fibronectin was first characterized as the large external transformation sensitive protein (LETS) because it is lost from the surface of transformed cells [32,33]. Some tumor cells lose their ability to attach to fibronectin after transformation [34]; this may be the result of a transformation-associated loss of the fibronectin receptor, integrin α5β1, from the cell surface [35] or, alternatively, could be caused by inactivation of the integrin α5β1 via a phosphorylation event [36].

Integrin α5β1 expression and tumor growth

A role for integrin α5β1 in the regulation of proliferation of tumor cells was initially suggested by a series of studies of tumor variants which overexpress α5β1. MG63 osteosarcoma cells [37,38] and K562 erythroleukemia cells [39] that were selected for an increased ability to attach to fibronectin exhibited a fivefold upregulation of α5β1 expression concomitant with significantly reduced anchorage-independent growth and tumorigenicity. The direct induction of tumor cell growth inhibition by integrin α5β1 expression was demonstrated when transfection of Chinese hamster ovary cells with the integrin α5 and β1 subunit genes resulted in cells that expressed 30-fold more α5β1 and showed a loss of tumorigenicity and reduced proliferation *in vitro* [40]. These results also suggested that the degree of growth inhibition is dependent on the level of α5β1 expression on the cell surface. In additional studies, loss of integrin α5β1 expression on Chinese hamster ovary cells led to enhanced tumorigenicity [41]. These findings document that integrin α5β1 is implicated in the growth regulation of tumor cells.

Recently, Varner *et al.* [5•] expressed the integrin α5β1 in HT29 colon carcinoma cells which normally lack α5β1. After being transfected with a cDNA encoding the α5 integrin subunit, these cells gained the ability to adhere to fibronectin. Interestingly, in the absence of a fibronectin substrate, expression of integrin α5β1 leads to a dominant-negative regulation of cellular proliferation [5•]. Integrin α5 transfected cells were either nontumorigenic or significantly less tumorigenic than control transfectants and parental tumor cells, and they proliferated at half the rate of control transfectants under anchorage-independent culture conditions. This growth suppression is associated with a failure to enter S phase, as monitored by thymidine incorporation into DNA, and with an upregulation of transcription of the growth arrest inducing gene *gas-1* [42,43] and a downregulation of transcription of the immediate early genes *c-fos*, *c-jun* and *jun B*. Ligation with fibronectin reverses the inhibition of proliferation, inhibits transcription of *gas-1* and induces transcription of the immediate early genes, in a tyrosine phosphorylation dependent manner [5•]. Although no studies have indicated a role for other fibronectin receptors in the negative regulation of tumor growth, it remains unclear whether or not alternative fibronectin receptors could suppress tumor cell proliferation or whether this is a unique property of the ectodomain and/or cytoplasmic tail regions of the α5β1 integrin.

Distinct integrins influence the biology of various tumor types

Expression of other integrin subunits, in particular α2β1 and αvβ3, also influences cellular proliferation and differentiation. The loss of expression of the integrin α2β1 in breast epithelial cells is correlated with the transformed phenotype [44•]. Antisense mRNA reduction of α2β1 levels in breast carcinoma cells induces a transformed phenotype [45]. In addition, the ectopic expression of integrin α2β1, a receptor for laminin and collagen, has been shown to suppress the growth of breast carcinoma cells and to induce their differentiation [44•]. Expression of this integrin altered the phenotype of poorly differentiated human and mouse breast carcinoma cells from a fibroblastoid, spindle-shaped phenotype to an epithelioid, polygonal-shaped, contact-inhibited phenotype. These transfected cells were then able to form glandular structures in three-dimensional matrices.

In addition, a novel alternatively spliced integrin β1 subunit, β1C, has recently been described [46••]. This molecule is a growth inhibitory subunit which prevents cell cycle progression [46••]. This progression is dependent on an amino acid sequence in its cytoplasmic domain that is located between amino acids 795 and 802 [47•].

In contrast, expression of some integrins positively regulates tumor cell proliferation. Expression of the integrin αvβ3 in metastatic, but not benign, melanomas [48,49] suggests a role for this integrin in the regulation of tumor proliferation. When melanoma cells were selected for loss of the αv integrin subunit, these cells exhibited significantly reduced proliferation and tumorigenicity which could be restored by re-expression of the integrin [50,51]. In further support of a role for αv integrin in tumor cell proliferation are studies in which antibody antagonists of the αv subunit prevented human melanoma tumor formation in nude mice [52].

Expression of the integrin subunits α6 and α3 is also associated with transformation and tumor progression. Integrin α3β1 is expressed in 82% of metastatic tumors [53]. Integrin α6 is expressed at increased levels in tumors of the head and neck [54], and in bladder cancer [55], lung

cancer [56] and colon carcinoma (JA Varner, unpublished data).

The molecular mechanisms by which these integrins regulate tumor cell growth are not clear at present, but it is likely that integrin signaling plays a central role in the process. Recently, a novel oncoprotein with tyrosine kinase activity that directly interacts with the integrin β1 cytoplasmic tail was described [57•]. The interactions of this kinase, called integrin-linked kinase-1 (ILK-1), and of other such signal transduction mediators may play important roles in integrin-regulated cellular proliferation. Thus, the pattern of integrin expression in the tumor cell is implicated in the enhanced proliferation that is a characteristic of tumor cells.

Regulation of apoptosis by integrins

Cellular attachment of epithelial, endothelial and some tumor cells to the extracellular matrix through integrins (or integrin cross-linking) promotes cell survival by inhibiting apoptosis, as determined by evaluation of DNA laddering, cellular morphology and presence of free 3′-hydroxyl groups [8–10,11•,58]. In fact, *de novo* expression of αvβ3 on human melanoma cells facilitated the increased survival of the cells in three-dimensional dermal collagen [9]. In addition, integrin ligation has been shown to regulate the expression of Bcl-2, a key regulatory component in the suppression of apoptosis [59•]. Ligation of integrin α5β1 in α5-transfected tumor cells (Chinese hamster ovary tumor cells), which exhibit reduced proliferation as compared with untransfected cells, prevented apoptosis by inducing Bcl-2 expression [60•]. Ligation of integrin αvβ3 in endothelial cells suppresses p53 activity, inhibits p21WAF1/CIP1 expression and increases the Bcl-2: Bax ratio, promoting cell survival [59•–61•]. In contrast, blocking integrin αvβ3 ligation with integrin antagonists induced p53 activation and blocked Bcl-2 expression [60•]. Interestingly, expression of the β4 cytoplasmic domain in cells activates p21 and induces growth arrest [61•].

Integrins in invasion and motility

Integrins also contribute to cellular motility and metastasis. For example, the integrin α2β1, a collagen/laminin receptor, has been shown to impart metastatic abilities to some tumor cells [62]. Integrin αvβ3, the most promiscuous member of the integrin family, mediates cellular adhesion to vitronectin, fibronectin, fibrinogen, laminin, collagen, von Willibrand factor, osteopontin, and adenovirus penton base, among other proteins [63–65]. Expression of this integrin enables a given cell to adhere to, migrate on, or respond to almost any matrix protein it may encounter. This migratory capacity is dependent on an intact NPXY (single-letter code for amino acids) sequence present within the integrin β3 subunit cytoplasmic tail [66]. Tumor cells transfected with a β3 cDNA containing a mutated NPXY sequence are unable to metastasize, in contrast to tumor cells transfected with an intact β3 subunit [66]. This integrin is expressed on migratory cells such as metastatic melanoma cells [48], in which its expression correlates with a role in metastasis [66,67]. An additional αv integrin, the integrin αvβ5, also directs tumor cell motility, but unlike αvβ3-mediated motility, αvβ5-mediated motility is dependent on receptor tyrosine kinase activity [68] and NF-κB-mediated gene expression [31].

Recently, the association of integrins and matrix metalloproteinases (MMPs) has been described. Recent studies by Brooks *et al.* [69•] demonstrated that the collagenase MMP-2 binds directly to integrin αvβ3 and is thus localized, in a proteolytically active form, on the surface of invasive tumor cells or endothelial cells. This localization appears to provide migratory cells with coordinated matrix degradation and cellular motility, thus facilitating cellular invasion processes [69•]. Furthermore, an association between integrin α2β1 and the positive regulation of MMP-1 expression has also been recently described [70], as has an association between integrin α5β1 and α4β1 ligation and metalloproteinase expression [71].

Role of integrins in tumor angiogenesis

Perhaps the most significant of the physiological roles played by integrin αvβ3 in cancer is its critical role in the process of angiogenesis. Integrin αvβ3 is minimally, if at all, expressed on resting, or normal, blood vessels, but is significantly upregulated on vascular cells within human tumors [10,72] and in response to growth factors *in vitro* [73,74] and *in vivo* [72,75]. For example, basic fibroblast growth factor (bFGF), but not transforming growth factor-β or interferon-γ, markedly increases β3 mRNA levels and β3 protein surface expression in cultured human dermal microvascular endothelial cells [73,74]. bFGF and tumor necrosis factor-α stimulate αvβ3 expression on developing blood vessels in the chick chorioallantoic membrane (CAM) and on the rabbit cornea [72,75]. Peak levels of integrin expression are observed on blood vessels 12–24 hours after stimulation with bFGF (our unpublished data). αvβ3 expression is also induced by human tumors cultured on the chick CAM [72,75] and by human tumors grown in human skin explants grafted onto SCID mice [76].

Antagonists of αvβ3 integrin promote tumor regression by disrupting angiogenesis

The highly restricted expression of αvβ3 integrin and the upregulation of its expression during angiogenesis suggest that it may play a critical role in the angiogenic process. In fact, recent experimental evidence supports this notion. Specifically, antagonists of integrin αvβ3, but not of β1 integrins, potently inhibit angiogenesis in a number of animal models. When angiogenesis is induced on the chick CAM with purified cytokines, αvβ3 expression is stimulated by fourfold within 72 hours [72]. Topical or systemic administration of LM609, a monoclonal antibody antagonist of αvβ3, inhibited angiogenesis, whereas other anti-integrin antibodies were ineffective [72]. Similarly, administration of LM609 or of a cyclic RGD peptide of

αvβ3 antagonists, but not of other anti-integrin antibodies or of control peptides, reduced the growth of blood vessels into tumors growing on the surface of CAMs. Importantly, LM609 had no effect on pre-existing vessels [72]. These findings suggest that αvβ3 plays a biological role in a critical event of blood vessel formation during tumor angiogenesis. Antagonists of integrin αvβ3 not only prevent the growth of tumor-associated blood vessels but this results in the regression of established tumors *in vivo* [10]. Histological examination of the anti-αvβ3-treated and control-treated tumors revealed that few, if any, viable tumor cells remained in the anti-αvβ3 treated tumors [10]. In fact, these treated tumors contained no viable blood vessels.

It is important that antagonists of integrin αvβ3 also inhibit tumor growth in human skin. In studies of the effect of these antagonists on human angiogenesis, Brooks *et al.* [76] transplanted human neonatal foreskins onto SCID mice. After permitting the skin to heal, they were able to demonstrate that the majority of the blood vessels within the human skin were human in origin. Human breast cancer tumors (αvβ3-negative) were established in the human skin transplants on these animals. Two weeks later, the mice were treated intravenously with LM609 or control antibodies. Tumor growth was either completely suppressed (in 8 out of 12 mice) or was significantly inhibited as compared with mice treated with a control antibody. Angiogenesis was significantly inhibited (by at least 75%) in the LM609-treated animals. Thus, LM609 appears to be effective in regulating the human angiogenic response to human tumors growing in a human tissue.

Importantly, not only did the LM609-treated animals contain smaller tumors but the tumours also appeared considerably less malignant than tumors in control animals; specifically, their margins were well defined, showing no evidence of tumor cell invasion [76]. In addition, there were fewer proliferative tumor cells in the LM609-treated animals. This was associated with a sharp decrease in the blood vessel counts in these tumors. Thus, by blocking tumor-induced angiogenesis it was possible to curtail the invasive or malignant properties of the tumor.

αvβ3 integrin regulates vascular cell survival *in vivo*

The mechanism of action of αvβ3 antagonists in blocking angiogenesis appears to be related to their ability to selectively promote unscheduled programmed cell death (apoptosis) of newly sprouting blood vessels, on the basis of increased DNA laddering and ApopTag staining for the presence of free 3′-hydroxyl groups in tissues treated with integrin αvβ3 antagonists [10]. To further evaluate the effects of these antagonists on vascular cell events, single-cell suspensions were prepared from CAMs treated with bFGF and in the presence or absence of LM609. These cells were then stained with the DNA dye propidium iodide to examine the DNA content per cell. Cells with greater than one copy of DNA were presumed to have entered the cell cycle. These cells were then costained with ApopTag to evaluate their degree of DNA breakdown. This costaining procedure revealed that bFGF could promote cell entry into the cell cycle and that LM609 caused ApopTag staining of these same cells. These findings demonstrated that the monoclonal antibody LM609 was capable of inducing apoptosis of vascular cells that had already responded to the cytokine [10], suggesting that αvβ3 promotes a survival signal critical for cells completing the cell cycle.

More importantly, these findings demonstrate that antagonists of αvβ3 integrin disrupt a stage of angiogenesis that occurs after induction but prior to vessel maturation. This is consistent with the studies by Drake *et al.* [77] showing that antagonists of αvβ3 integrin blocked late-stage development of new blood vessels in the quail by preventing lumen formation. Together, these findings are consistent with the notion that αvβ3 provides a survival signal to proliferative vascular cells during new blood vessel growth. Presumably, after new blood vessels are fully mature, the vascular cells are refractory to antagonists of this integrin. These findings may explain why antagonists of αvβ3 selectively impact newly growing blood vessels. It is not currently known if integrin αvβ5 antagonists also induce apoptosis in angiogenic blood vessels.

Angiogenesis depends both upon the stimulation of quiescent vascular cells by growth factors released from tumors or other diseased tissues and also upon the interaction of the integrins αvβ3 and αvβ5 with one of their ligands [72,75]. Stimulated endothelial cells depend on integrin function for survival during a critical period of the angiogenic process, as inhibition of αvβ3–ligand interaction by antibody or peptide antagonists induces vascular cell apoptosis and inhibits angiogenesis [72,75].

Conclusions

Recent published reports have documented a significant role for integrins in the regulation of tumor cell survival, proliferation and invasion. Importantly, tumor cell growth and malignant behavior also depend on angiogenesis, a process that depends on the endothelial cell αvβ3 integrin.

Future studies are likely to focus on integrin-mediated signaling and cell biological events that contribute to the malignant behavior of solid tumors.

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